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DESIGN AND CONSTRUCTION OF A SMALL WHOLE BODY INHALATION CHAMBER

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ABSTRACT

An animal model whole body inhalation study of carbon monoxide gas necessitated the development of an exposure chamber and suitable caging for both the exposure and subsequent treatment with hyperbaric oxygen of test animals. The exposure chamber was constructed from both polycarbonate and acrylic plastics. It uses a plenum design for both the input and the exhaust systems to assure uniform distribution of the carbon monoxide vapors within the exposure chamber. This chamber design is also suitable for use with other gases provided they are chemically compatible with acrylic, and polycarbonate.

KEY WORDS AND PHRASES

Carbon Monoxide, Inhalation, Analyzer, Plenum, Polycarbonate, Acrylic, Vapor Exposure, Animal Model.

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INTRODUCTION

A small whole body exposure chamber capable of handling up to 16 animals was required to conduct carbon monoxide (CO) exposures. The chamber was required to give a uniform distribution of CO throughout the exposure volume, and to consistently maintain exposure levels over long periods of time with minimum adjustments. It was anticipated that carbon monoxide exposures ranging in concentrations of 1000-3000 ppm would be used and the exposures times could last for periods of several hours. The chamber was constructed with a top and bottom plenum area. This architecture allowed for a uniform distribution of CO input into the chamber and uniform evacuation of the respired gases along with the excess CO from the chamber. The large volume of the upper plenum helps to average out minor fluctuations in the input stream resulting in a stable operation over long periods of time. Post exposure treatment of the animals would be conducted in a hyperbaric chamber (Type II, Animal hyperbaric chamber, #33629, Dixie Manufacturing). The internal length and diameter of this hyperbaric chamber is such that it held two Toxic Hazard Research Unit (THRU) animal cages. Therefore, the exposure chamber was sized to accept two of these cages.

The exposure chamber was built entirely from $^{3}/_{8}$ inch polycarbonate plastic with the exception of the cap of the bottom plenum which was fabricated using $^{3}/_{8}$ inch acrylic plastic. The cap on the bottom plenum serves both for exhaust flow and support for the animal cages. Four pulls were added to this cap to facilitate its removal from the chamber for cleaning of the bottom plenum cavity. Polycarbonate has several desirable properties. First, it is readily sawn, drilled and routed. Secondly it can be solvent welded to itself. Third it has a degree of flexibility which makes it highly shatter resistant. However, the bottom plenum cap was made from $^{3}/_{8}$ inch acrylic for this application because it is more rigid than polycarbonate, thereby giving better support to the cages. In addition, because of its stiffness acrylic also achieves a better seal with the lower plenum flange.

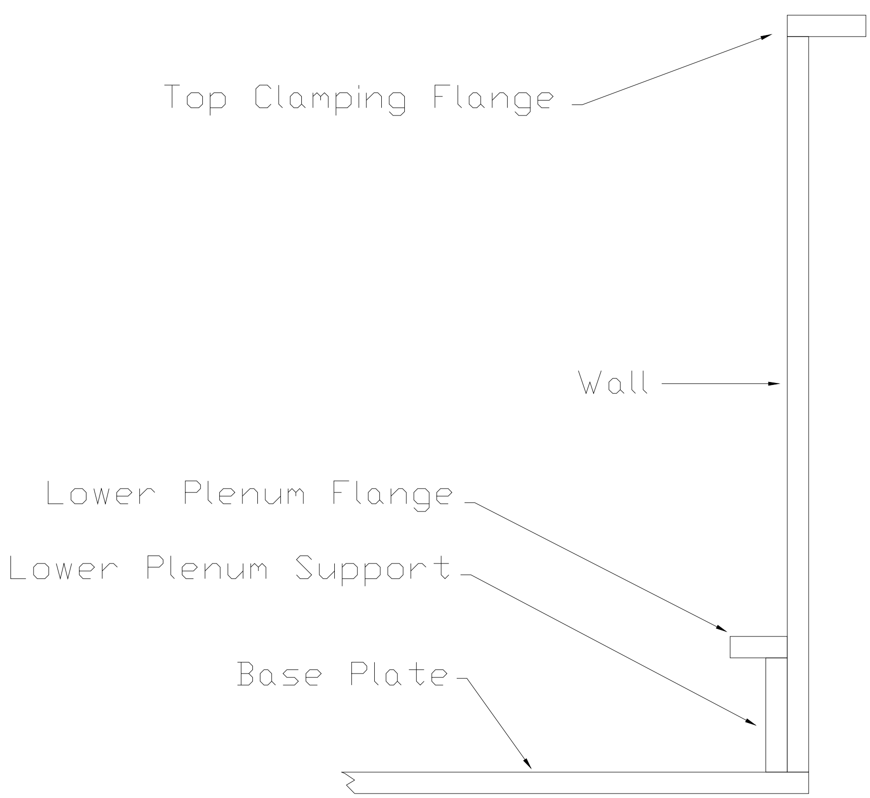
Methods

Base and Flange Construction

A $^{3}/_{8}$ inch thick by 30 $^{5}/_{8}$ inch by 28 $^{11}/_{16}$ inch plate of polycarbonate was cut using a table saw (Rigid, Model TS3650) equipped with a 10 inch non-melting plastics blade (MTC, model LB10801, Amana Tools) to serve as the base of the exposure box. The side walls ($^{3}/_{8}$ inch thick by 12 $^{7}/_{8}$ inch high) were solvent welded onto this base using Weldon 4 (Ridout Plastics, San Diego CA). On the inside of each side wall a 2 inch high x $^{3}/_{8}$ inch thick strip of polycarbonate was solvent welded to serve as a support for an internal flange. On top of this support a 1 inch wide by $^{3}/_{8}$ inch thick polycarbonate flange was solvent welded (Figure 1). This flange serves as a support for the acrylic lower plenum cap.

A 1 inch wide neoprene rubber gasket was placed on the top of this flange. Vacuum grease was applied to both sides of the gasket to enhance the seal between it and the acrylic cap. The gasket was prepared by cutting a 28 inch by 30 inch rectangle from a neoprene rubber sheet. A one inch border was drawn on this rectangle and the center rectangle removed leaving the finished gasket.
On the top of the walls, a one inch wide flange was solvent welded to serve as a clamping area to secure the lid of the exposure chamber to the bottom. The flange was welded flush with the insides of the side walls to create a $^5/_8$ inch overhang. On top of this flange $^1/_2$ inch wide by $^1/_4$ inch thick sticky backed weather stripping was added (Figure 1).

Figure 1

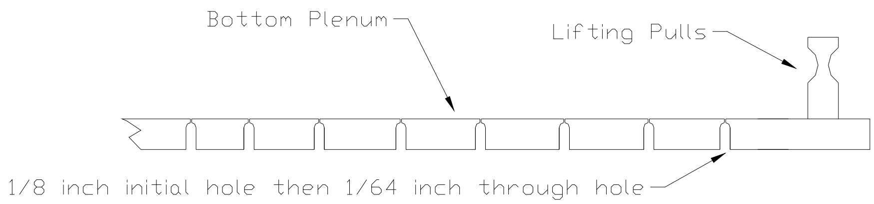


Plenum Construction

A 28 inch by 30 inch $^3/_8$ inch thick acrylic rectangle was cut from a larger piece of acrylic sheet on the table saw. To create the plenum this sheet was drilled with 81 holes measuring $^1/_{64}$ inch in diameter. The holes were laid out in a 9 by 9 grid uniformly spaced over the surface of the sheet. The holes started and ended approximately 2 $^3/_4$

inches from the edges. Because of the thickness of the plastic it was not practical to drill the $^{1}/_{64}$ inch holes directly. Therefore, $^{1}/_{8}$ inch holes were drilled 90% of the way through the plastic using a brad point bit (DeWalt drills). Both to assure that the holes were drilled perpendicular and to control the depth of the hole, a hand held drill guide was used (Portalign, out of production, Equivalent Craftsman Drill Guide, Sears). The remaining 10% was drilled out with a $^{1}/_{64}$ inch bit hand held in a pin vise. The holes were oriented so that the larger opening faced down into the plenum area (Figure 2).

Figure 2

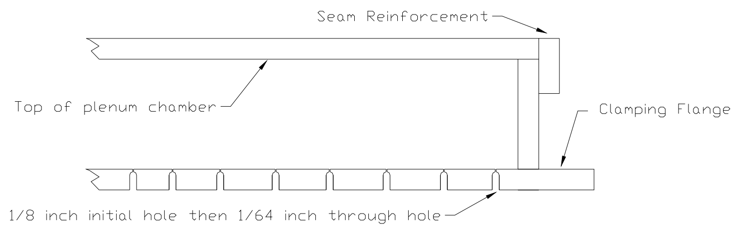


The acrylic sheet was then pressed onto a neoprene gasket on the lower plenum flange of the chamber creating the lower plenum. To help accommodate removal of this piece, four lifting pulls were constructed from $^{3}/_{8}$ inch polycarbonate. Strips 1 inch long by 1 $^{1}/_{8}$ inch tall were cut and a $^{1}/_{2}$ inch by $^{3}/_{16}$ inch deep groove was routed on both sides just below the top edge using a $^{1}/_{2}$ inch core box router bit (Woodriver corebox, Woodcraft, Parkersburg WV). These pieces were solvent welded to the acrylic sheet in each corner (Figure 2).

The top of the chamber was started by cutting out a 32 inch by 30 inch plate of $^{3}/_{8}$ inch polycarbonate. This was drilled out with the same pattern as was used for the lower plenum. Every other hole in this plenum plate was then opened up to $^{1}/_{32}$ inch to reduce the pressure to flow relationship in this plenum. A $^{3}/_{8}$ inch thick by 28 $^{3}/_{4}$ by 30 $^{3}/_{4}$ by 2 inch high polycarbonate rectangle was solvent welded together. This rectangle was centered and solvent welded onto the upper plenum plate. Next a 28 $^{3}/_{4}$ by 30 $^{3}/_{4}$ by $^{3}/_{8}$ inch thick polycarbonate plate was solvent welded onto the top of the rectangle. Finally, a 1 inch wide band of $^{3}/_{8}$ inch polycarbonate was solvent welded over the seam between the top plate and sidewalls (Figure 3). The last step was to run a small tipped soldering iron along all the seams in the entire exposure box and to melt these seams together to a depth of approximately $^{1}/_{16}$ of an inch.

The top and bottom of the chamber were held together using one inch spring clamps (Just Clamps, model 616,Atlanta Ga) on their respective flanges. These clamps were spaced evenly around the periphery of the chamber, to assure a uniform seal.

Figure 3



Analysis of CO Distribution

The uniformity of distribution of CO was determined by analyzing the concentration of CO at 6 positions in the exposure chamber relative to the center of the chamber (Figure 4). The exposure chamber was operated at 1000 ppm using the system shown in Figure 5. A timer (Gralab model 655, Gralab Corp, Centerville, OH) was programmed to operate in interval mode. The timer was connected to a 3 way solenoid which would alternately open one leg or the other under timer control. During one timer interval, a sample of 1000 ppm of CO was drawn from location C for sufficient time to achieve equilibrium as shown by the Binos (Inficon, Model 0091, East Syracuse NY) analyzer. During the next timer interval the solenoid switched to location 3 and the concentration was again analyzed by the Binos analyzer. After location 3 was analyzed location C was again analyzed. The probe was moved to location 2 and the process repeated until all 6 locations had been analyzed for CO concentration versus the concentration at location C.

Figure 4

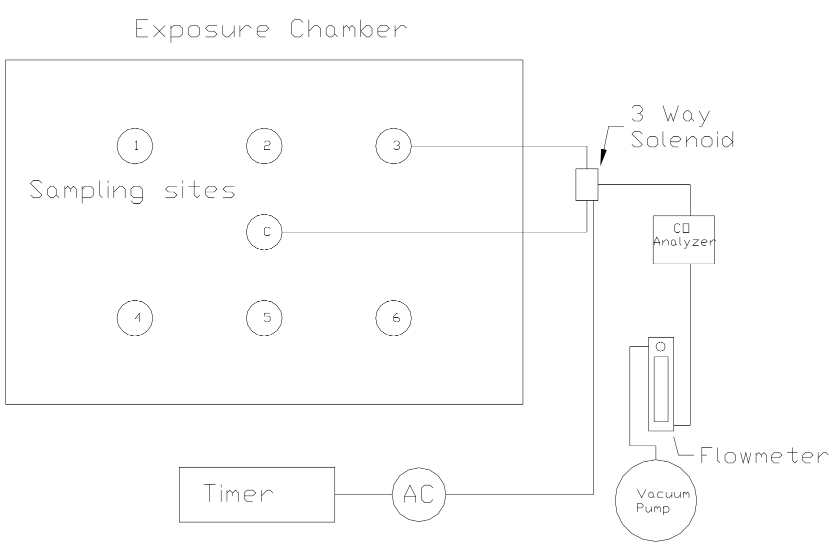
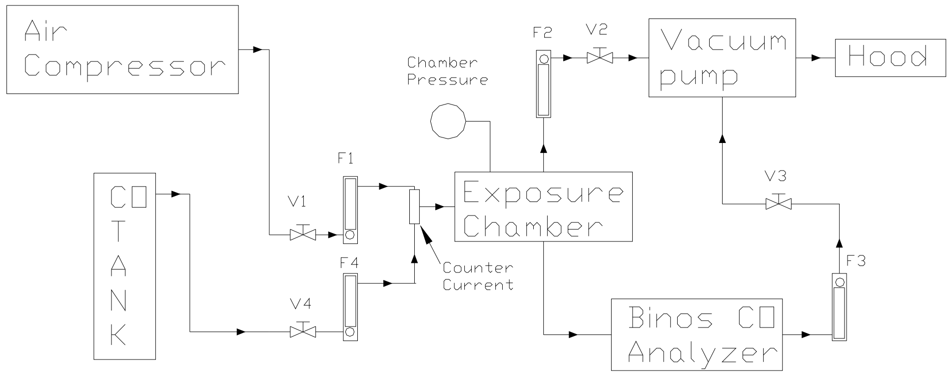


Figure 5



Chamber Operation

The exposure chamber was incorporated into a complete exposure system for testing. A tank of one percent (10,000ppm) of carbon monoxide in air was used for the toxicant. This gas was mixed in a 1 to 10 ratio with breathing quality compressed air to obtain a 1000 ppm mixture. The mixture ratio was controlled by adjusting the flows from two flow meters. Initial mixing of these gases was achieved by introducing the flows in an opposed fashion (counter current) into a tee fitting. The mixed gases were then passed into the upper plenum of the exposure chamber for dispersal into the exposure chamber. A vacuum system was connected to the lower plenum via another flow meter to control the exhaust flow. A $^1/_4$ inch stainless steel probe located in the center of the chamber was connected to a Binos CO analyzer via another flow meter to the vacuum system, to provide a real-time analysis of CO gas within the chamber. Calibration of the Binos analyzer was performed using standard bag methodology (Reboulet et. al, 2009).

RESULTS

Distribution of CO within the exposure chamber was extremely uniform with no variance at any of the 6 sampling locations relative to the center of the chamber.

The leak rate of the chamber was determined to be 56 ml/min with a chamber total volume of 206 liters using published methods of leak rate determination (Mokeler & White, 1983, Kimmel & Reboulet, 1998). This corresponds to a leakage of 0.02 %, which is well within the acceptable limits used by this laboratory of 0.1 % for a new chamber.

Operating the chamber in the range of 12 to 15 exchanges per hour, the CO concentration reached equilibrium in approximately 20 minutes as predicted using

Silver's equation (Silver, 1946). Once equilibrium was established the system was able to be operated stably for periods of several hours.

DISCUSSION

The exposure chamber was sized to hold two THRU cages which would hold 8 rats each and also would fit into the hyperbaric chamber to be used for post exposure treatment of the animals. A number of technical difficulties arose in the construction of the exposure chamber. Even though, a special plastic cutting saw blade was used the seemingly smooth edges on the plastic did not provide an airtight seal at the seams after solvent welding. Filling the box with water revealed numerous small areas of leakage. Re-solvent welding the areas of leakage was marginally successful. This problem was solved by using a hot soldering iron over the seams to completely seal them. These leakage issues could have been minimized if a router were used to smooth the edges prior to solvent welding

During the validation process it was apparent that the pressure in the upper plenum was far too great to achieve 15 exchanges of atmosphere per hour. Therefore, every other hole was opened up from its initial $^{1}/_{64}$ inch diameter to $^{1}/_{32}$ inch diameter to reduce this pressure. Even at this reduced pressure there was consequent flexion of the lid during operation which resulted in undue stresses on the upper seam. To mitigate this problem a 1 inch wide band of $^{3}/_{8}$ inch polycarbonate was added to reinforce this seam.

Sealing the chamber lid to the bottom by using small clamps on the flanges was found to be a workable solution to seal the chamber. However, the process of clamping and unclamping is time consuming and prone to operator error. Insufficient time was available prior to the start of the experiments to make any additional modifications to the chamber. In a future modification the clamping flanges would be replaced with linear pull latches, a pressure gauge would be inserted into the upper plenum area and additional holes in the upper plenum would be opened to $^{1}/_{32}$ inches to achieve the best pressure to flow rate ratio. While this chamber was developed for carbon monoxide exposures, other gases and combinations of gases could also be used. However, any material used would have to be non-reactive to polycarbonate and acrylic.

CONCLUSION

The exposure chamber met its design criterion. It allowed exposure of up to 16 animals at one time, gave a uniform distribution of the CO gases, and was stable in operation for periods of several hours. Several modifications would improve the construction and operation of this exposure chamber. It could also serve as an exposure chamber for other gases and combinations of gases but not aerosols.

REFERENCES

1. Reboulet, James, Cunningham, Robert, Gunasekar, Palur G., Chapman, Gail D. and Stevens, Sean C. 2009. Loop System for Creating Jet Fuel Vapor Standards Used in the Calibration of Infrared Spectrophotometers and Gas Chromatographs. Toxicology Mechanisms and Methods. 19:2,123 – 128

2.Mokler, B. V. and R. K. White. 1983. Quantitative standard for exposure chamber integrity. Am. Ind. Hyg. Assoc. J**.** 44:292-295.

3. Kimmel E.C. and Reboulet J.E. 1998. Calculation of Exposure Chamber Leak Rate with Thermal Correction: A Measure of Chamber Integrity. Am. Ind. Hyg. J. 59:11,779-784

4. Silver S.D. 1946. Constant Flow Gassing Chambers: Principles Influencing Designs and Operation. J. Lab. Chem. Med. 31:1153-1161